31

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/54, C12Q 1/68, C07K 16/40, C12N 9/12

A1

(11) International Publication Number: WO 96/11275

(43) International Publication Date: 18 April 1996 (18.04.96)

(21) International Application Number: PCT/FI95/00555

(22) International Filing Date: 9 October 1995 (09.10.95)

(30) Priority Data:
08/320,432 7 October 1994 (07.10.94) US

(71) Applicant: HELSINKI UNIVERSITY LICENSING LTD. OY [FI/FI]; Teollisuuskatu 23, FIN-00510 Helsinki (FI).

(72) Inventor: ALITALO, Kari; Nyyrikintie 4A, FIN-02100 Espoo (FI).

(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6A, FIN-00120 Helsinki (FI).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CYTOPLASMIC TYROSINE KINASE

(57) Abstract

Provided are cytoplasmic tyrosine kinase molecules, DNA encoding them; and methods for their use and production.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

CYTOPLASMIC TYROSINE KINASE

FIELD OF THE INVENTION

The present invention generally relates to a novel cytoplasmic tyrosine kinase, the gene encoding it, and its use in diagnostic and therapeutic procedures.

BACKGROUND OF THE INVENTION

Cellular processes involved in the maintenance, differentiation, and repair of cells and tissues are regulated, in part, by intercellular and intracellular signals which are controlled by the binding of growth factors and other ligands to their receptors. One important mode of signalling used by cells to regulate gene expression and activation or deactivation of biochemical pathways is tyrosine phosphorylation. Numerous tyrosine kinases are known and they usually exist as transmembrane receptors for polypeptide growth factors, such as epidermal growth factor, insulin, insulin-like growth factor I,platelet-derived growth factors, and fibroblast growth factors. *See*, generally, Ullrich, *et al.*, *Cell*, *61*:243-254 (1990); and Heldin, *et al.*, *Cell Regulation*, *1*:555-556 (1990). Of interest to the present invention are several receptor tyrosine kinases which recognize hematopoietic growth factors as their ligands. These include the c-fms receptor tyrosine kinase which is the receptor for colony-stimulating factor 1, Sherr, *et al.*, *Cell 41*: 665-676 (1985), and c-kit, a primitive and less well-characterized hematopoietic growth factor reported in Huang, *et al.*, *Cell 63*: 225-233 (1990).

Tyrosine kinases are generally divided into families, subfamilies, and classes based upon structural characteristics. In general, two broad classifications exist and those include membrane-bound tyrosine receptor kinases and nonreceptor tyrosine kinases, which include cytoplasmic and nuclear tyrosine kinases. An example of tyrosine receptor kinases is the family of epidermal growth factor receptor kinases which form a subfamily of transmembrane receptor kinases with extracellular domains. That subfamily, along with others, such as insulin receptor kinases, contain homologous cysteine-rich repeats in their extracellular domains. *See* Hirai, *et al.*, *Science, 238*: 1717-1720 (1987). Other membrane-bound receptor tyrosine kinases contain extracellular folds which are characteristic of the immunoglobulin superfamily.

The nonreceptor tyrosine kinases are often referred to as a single group comprising Src-like kinases, but it is now recognized that the nonreceptor tyrosine kinases may be divided into several families. Bolen, *Oncogene, 8*: 2025-2031 (1993); Wang, *TIBS 19*, 373-376, 1994.

For example, a newly-identified non-receptor tyrosine kinase family includes three independently-cloned genes, *TEC*, *ITK* (also known as *TSK* or *EMT*), and *BTK* (formerly known as *ATK*, or *EMB*). Proteins encoded by those genes are generally homologous to the *Drosophila melanogaster* Src28C tyrosine kinase. Such peptides generally contain SH3 and SH2 domains upstream of the tyrosine kinase domain. However, peptides encoded by *TEC/ITK/BTK* also typically contain a long N-terminal region which does not have a consensus myristylation

residue which is generally conserved in Src-like kinases. Instead, the N-terminal regions of proteins of the *TEC/ITK/BTK* family contain a pleckstrin homology (PH) domain which is described in Musacchio, *et al.*, *TIBS, 18*: 343-348 (1993). Finally, the *TEC/ITK/BTK* family generally consists of peptides which have a short C-terminus, lacking the regulatory tyrosine phosphorylation site found in most Src-like kinases.

The core of the pleckstrin domain is an antiparallel beta-sheet consisting of seven strands. The C-terminus is folded into a long alpha-helix. The domain is electrostatically polarized and contains a pocket which may be involved in binding to a ligand, such as a peptide or a small protein. This core structure is conserved in all PH domains, which, however, have large variations in the loops surrounding the putative binding pocket. The functions of pleckstrin domains are still unknown, although they have been shown to bind to the β and γ subunits of complex G-proteins.

The gene encoding the *TEC* tyrosine kinase was identified in murine hepatocarcinoma cells and was later found to be expressed in all murine hematopoietic cell lines examined. Mano, *et al.*, *Oncogene, 8*: 417-424 (1993). The other two members of the family, *ITK* and *BTK*, are selectively expressed at certain stages of T-cell and B-cell development, respectively. Expression of the *ITK* mRNA is induced upon T-cell activation by IL-2 and mutations in *BTK* are thought to be responsible for X-linked agammaglobulinemia (Burton's disease, XLA), a disease characterized by a lack of circulating mature B-cells in affected males. Several different BTK mutations have been described in murine models

of XLA, including point mutations in the PH or SH2 domains, which may be involved in functionally important interactions with other proteins.

Cytoplasmic tyrosine kinases have been reported to associate with ligand-activated transmembrane receptors and they appear to initiate or to amplify ligand-induced signals. For example, the T- and B-cell receptors and cytokine receptors in hematopoietic cells associate with certain members of the Src tyrosine kinase family in order to transduce their signals. Upon ligand binding to these receptors, a rapid increase in tyrosine phosphorylation of specific intracellular substrates is observed. These signalling pathways appear therefore to depend on specific intracellular tyrosine kinases recruited and activated by the stimulated receptors. Members of the Src family are expressed in a cell lineage-selective manner, which is consistent with this hypothesis. Several cytokine receptors also interact with and activate JAK family of kinases, which in turn directly phosphorylate transcriptional regulators.

In contrast to cytokine receptors, most growth factor receptors contain intrinsic tyrosine kinase activity. Autophosphorylated tyrosyl residues of some of these receptors, such as platelet-derived growth factor receptor and hepatocyte growth factor/scatter factor receptor bind to SH2 domains of cytoplasmic tyrosine kinases, such as c-Src. The recruitment of a cytoplasmic tyrosine kinase to an activated receptor complex can amplify the signal by associating with other SH2 domain-containing signal transducers and possibly with proteins binding to the SH3 domain.

The present invention provides a new cytoplasmic receptor tyrosine kinase which shares characteristics with members of the *TEC/ITK/BTK* subfamily and is useful as a marker for cell growth and differentiation and for various types of tumor formation and in the diagnostics and treatment of diseases resulting from deregulated tyrosine phosphorylation. Using a presently-claimed cytoplasmic tyrosine kinase, it is possible to isolate the growth factor or cytokine receptor whose signals are mediated through such kinases by methods standard in the art.

SUMMARY OF THE INVENTION

The present invention provides novel cytoplasmic tyrosine kinases capable of stimulating growth and/or proliferation of hematopoietic cells. In a preferred embodiment, a protein according to the invention is a *BMX* protein comprising the amino acid sequence shown in SEQ ID NO: 3. Also in a preferred embodiment, the invention provides cDNAs encoding *BMX*.

In a preferred embodiment, a *BMX* tyrosine kinase according to the invention comprises a fragment of the *BMX* protein which is capable of stimulating the growth and/or differentiation of hematopoietic cells, whether *in vivo* or *in vitro*. Also in a preferred embodiment, the invention provides a DNA encoding a fragment of the *BMX* protein, which fragment is capable of stimulating the growth and/or differentiation of hematopoietic cells.

The present invention also provides an antibody directed against proteins of the invention;

including a monoclonal antibody and a hybridoma producing it.

Methods according to the invention comprise means for detecting the growth and/or differentiation of hematopoietic cells comprising the steps of exposing tissue to a detectably-labelled DNA or antibody according to the invention; washing the tissue, and detecting any label which remains in the tissue after washing. Methods for labelling DNA and protein and methods for preparing tissue for hybridization and immunohistochemistry are well-known in the art. The present invention also provides a unique tyrosine kinase, the activity of which is inhibited by specific inhibitors with consequent effects on the growth or differentiation of cells expressing *BMX*.

Additional embodiments and features of the invention will become apparent to the ordinarily-skilled artisan upon consideration of the following detailed description thereof.

DESCRIPTION OF THE FIGURES

Figure 1 shows amino acid sequences of *BMX* (SEQ ID NO: 3), BTK (SEQ ID NO: 4), ITK (SEQ ID NO: 5), TEC (SEQ ID NO: 6), DSrc28C (SEQ ID NO: 8) and the consensus sequence (SEQ ID NO: 7).

Figure 2A is a Northern blot and hybridization analysis of polyA+ RNA from human umbilical vein endothelial cells (HUVEC) and HT-1080 human fibrosarcoma cells.

Figure 2B is a Western blot of anti-BMX and control anti-SEX immunoprecipitates from HUVEC cells.

Figure 2C shows anti-PTyr Western analysis of *BMX* immunoprecipitates from COS cells transfected

with a *BMX*-containing vector or an empty vector (MOCK).

Figure 2D shows SDS-PAGE analysis of immunoprecipitates from COS-transfected cells (*BMX*),subjected to in vitro kinase reaction.

Figure 3 is a Western blot of *BMX* retrovirus-expressing *BMX* or control virus-infected (c) NIH3T3 cells lysed directly (lanes marked "-") or immunoprecipitated (IP) using anti-*BMX* antiserum.

Figure 4 shows a normal metaphase human chromosomes and an enlargement of the X chromosome showing localization of the *BMX*-encoding gene, along with a schematic depiction of the chromosome.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel tyrosine kinases, such as the *BMX* tyrosine kinase shown in SEQ ID NO: 3; fragments of said kinases; and DNA encoding said kinases and said fragments. Such cDNAs were isolated from genomic libraries constructed from bone marrow and endothelial cell sources. The *BMX*-encoding cDNA comprises an open reading frame of 2025 bp, encoding 675 amino acids. The protein product comprises a single tyrosine kinase domain, an SH2 domain, and an SH3 domain. The tyrosine kinase domain is approximately 70% homologous to the tyrosine kinase domains of BTK, ITK, and TEC as shown by a comparison of SEQ ID NO: 3 with SEQ ID NO: 4, 5, and 6 respectively. A fragment according to the present invention is any portion of the primary structure of the intact kinase which retains the ability to stimulate or inhibit the growth and/or differentiation of hematopoietic cells.

A *BMX* cytoplasmic tyrosine kinase according to the invention has a deduced amino acid sequence, shown in SEQ ID NO: 3, which is closely homologous to the sequences of Btk, Itk, Tec, and *Drosophila melanogaster* Src28C tyrosine kinases. The alignment of the *BMX* sequence with its closest homologous sequences is shown in Figure 1. Inspection of that figure,suggests that the ATG codon at position 36 of the *BMX* cDNA is the translation initiation site. That codon is embedded in a kozak consensus translation initiation sequence (AATATGG).

It has been reported that the variable N-terminal domains of members of the Src family of kinases interact with transmembrane receptors. *Mustelin, et al., TIBS: 18*: 215-220 (1993). As shown in Figure 1, the N-terminal region of *BMX* has significant homology with those of *TEC*, *ITK*, and *BTK*. The N-terminal domain of *BMX* also has a region which compares to the PH (Pleckstrin Homology) consensus sequence found in various GPTase activating (GAP) proteins and in other kinases and cytosketetal proteins. The core of the pleckstrin domain is an antiparallel beta-sheet consisting of seven strands and the C-terminal portion is folded into a long alpha helix. The Pleckstrin domain is electrostatically polarized and contains a putative ligand-binding domain.

In contrast to cytokine receptors, most growth factor receptors contain intrinsic tyrosine kinase activity. Autophosphorylated tyrosyl residues of some growth factor receptors (*e.g.*, platelet-derived growth factor receptor) bind to SH2 domains of cytoplasmic tyrosine kinases, such as c-Src. The recruitment of a cytoplasmic tyrosine

kinases to an activated growth factor receptor complex amplifies the signal through that complex by association of the tyrosine kinase with other SH3 and SH2 containing signal transducers.

Claimed *BMX* proteins possess both SH2 and SH3 domains. However, the *BMX* SH3 domain varies from the consensus sequence in that it includes two strongly hydrophilic portions rich in Ser and Glu residues in its C-terminus. This distinction may be due to a splice variation. The foregoing provides substantial evidence that the inventive tyrosine kinases bind active portions of growth factor receptors and, due to the localization of claimed proteins, do so in hematopoietic cell lines.

Accordingly, inventive proteins are useful for stimulating hematopoietic cell lines. Moreover,inventive DNAs are useful as diagnostic reagents in the detection of hematopoietic cell proliferation and oncogenesis. Antibodies and peptides of the invention are additionally useful for inhibiting activity of growth factors. The following examples provide details of the isolation, characterization, localization, and use of inventive proteins, DNAs, and methods for use thereof.

EXAMPLE 1

Cloning and Analysis of cDNA Encoding Proteins of the Invention

Total RNA was prepared from normal human bone marrow by guanidium thiocyanate extraction. An aliquot of 2 μg RNA was then reversed-transcribed using 10U of avian myeloblastosis virus reverse transcriptase in the presence of 0.5 μg oligo-dT primer, 1 μM each of deoxyadenosine triphosphate, deoxyguanine triphosphate, deoxycytosine

triphosphate, and deoxythymidine triphosphate, and 10 U RNAsin (Promega, Madison, WI). The reaction buffer contained 50 mM Tris-HCl, pH8.1,6mM $MgCl_2$, 40 mM KCl and 1mM dithiothreitol. The reaction contents were incubated at 42°C for 1 hour, then at 52°C for 30 minutes, and then at 95°C for 5 minutes.

Approximately 3% of the reverse transcribed cDNA was then amplified by PCR in a reaction volume of 100 ml using 3 U Dynazyme (Finnzymes, Helsinki, FI) in a reaction buffer comprising 1.5 mM $MgCl_2$ and in the presence of 200 5 μm each of deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanine triphosphate, and deoxythymidine triphosphate. The primers were 5'-GGTCTAGAA(A/g)AA(A/G)TT(C/T)GT(C/G)CAC(A/C)G(G/A)GAC-3'(0.1 5m) (SEQ ID NO: 1) and 5'-GCTCTAGA(G/A)GGCCATCCA(T/C)TT(G/C/A)AC(T/C/A)GG-3' (0.15m) (SEQ ID NO: 2) which represented the sense and antisense primers, respectively. Both primers were obtained from conserved tyrosine kinase domains from known kinases. The protocol for amplification was 90 seconds at 95°C; 120 seconds at 42°C; 180 seconds at 68°C for 35 cycles in a volume of 100 μl in a Perkin Elmer DNA Thermo Cycler 480. A 150 bp cDNA product representing the novel *BMX*-encoding sequences was obtained. That product was subcloned into a pCR vector using a TA-cloning kit (Invitrogen) according to the Manufacturer's instruction.

The PCR-amplified *BMX* product designated B1, described above, was radiolabelled with 32pCTP using random priming and used to screen an oligo-dT-primed λgt10 cDNA library constructed from human bone marrow RNA (Clontech). The B1 cDNA

included the sequence obtained by PCR amplification and flanking open reading frame, predicting a cytoplasmic tyrosine kinase very closely related to the newly identified Btk kinase. When the B1 cDNA was used to probe Northern blots, no specific signal was detected in any cell lines examined. Nevertheless, according to results obtained by reverse transcription-PCR amplification of RNA, *BMX* appeared to be expressed, not only in bone marrow, but also in endothelial cells. Therefore, a human cDNA library derived from endothelial cell RNA was screened in order to obtain full length cDNA. Several positive plaques were chosen and the longest *BMX* cDNA insert of approximately 2.4 kb (E7) was subcloned into a pGEM plasmid (Promega) and sequenced on both strands using the dideoxy chain termination method of Sanger. The E7 clone was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 on October 5, 1994 as accession No. ATCC 75907 . Computer analyses of the sequences were performed using the GCG program as reported in Devereux, *et al., Nucl. Acids Res., 12*: 387-395 (1984), incorporated by reference herein. The *BMX* sequence obtained is shown in Figure 1 and in SEQ ID NO: 3.

The E7 subclone contained an open reading frame capable of encoding 675 amino acids. The deduced amino acid sequence was closely homologous to the sequences of TEC, ITK, and BTK and to the *Drosophila melanogaster* Src28C tyrosine kinase. Sequence alignment suggested that the ATG at position 36 of the cDNA was the translation initiation site.

The *BMX* protein has an N-terminal region of 210 residues comprising a PH domain (shaded

region in Fig. 1), but no consensus myristylation site. The variable amino terminal domains of the Src family are involved in interaction with transmembrane receptors. Accordingly, it is interesting to note that the long N-terminal region of *BMX* shares a high degree of homology with the *Btk*, *Itk* and *Tec* kinases. This part of the molecule may have a role in associating with as yet unknown receptors or signal transducers. In this region, the sequences of these four tyrosine kinases are rich in basic amino acid residues and they fit the consensus for the PH domain found in a number of proteins, such as certain GTPase activating proteins (GAP) and GDP-GTP exchange factors (such as SOS1), in kinases such as sARK and RAC, and in dynamin, kinesin and spectrin.

The PH domain is followed by an SH3 and an SH2 domain (boxed region in Figure 1). For comparison, the corresponding regions of *Drosophila melanogaster* Src28C TK sequence are also shown in Figure 1. The sequences between amino acid residues 185-206 and 207-228 (horizontal arrows in Figure 1) of *BMX* are about 80% identical with each other both at the nucleotide and at the amino acid level, suggesting that this stretch originated from a duplication of the *BMX* DNA sequence. The latter stretch (207-228) belongs to the N-terminal portion of the *BMX* SH3 domain.

Although features of SH3 domains are common to all members of the *BMX/BTK/ITK/TEC* tyrosine kinase family, some of the sequences diverge from the consensus. The C-terminal portion of the *BMX* SH3 sequence (downstream of the WW motif at position 243) diverges from those of the other kinases, and contains two strongly hydrophilic

stretches, rich in Ser and Glu residues. In contrast, the SH2 domain is well-conserved in the *BMX* sequence when compared with the other tyrosine kinases (Fig. 1). Within the tyrosine kinase domain (arrows) the ATP binding motif containing the Gly(434)XGlyXXGly sequence and the Lys435 residue are marked in Fig 1. The Tyr566 residue of *BMX* corresponds to the conserved Tyr416 autophosphorylation site of c-Src. The catalytic domain is followed by a short C-terminal tail, where nonconserved autophosphorylation sites are found. Multiple stop codons were found after codon 675 of the *BMX* sequence.

Polyadenylated RNAs from several human fetal and adult tissues were analyzed for *BMX* RNA by Northern blotting and hybridization. *BMX* transcripts were prominent in both fetal and adult heart. Weaker signals were obtained from fetal lung and kidney; and from adult skeletal muscle, placenta, lung, liver, testis, ovary, and small and large intestine. Adult kidney, pancreas and prostate gave signals only after a very long exposure of the autoradiogram, whereas no signal could be obtained from fetal or adult brain or fetal liver or kidney. Thus, *BMX* appears to be more widely expressed than the related Btk, Itk and Tec tyrosine kinases. At least part of these hybridization signals may be derived from hematopoietic cells in these organs.

EXAMPLE 2

Expression and Analysis of the *BMX* Protein

A *BMX* retrovirus was constructed into pBABEpuro vector. The pBABEpuro vector is reported in Morgenstern, *et al., Nucl. Acids Res., 12*: 387-395 (1990). The resulting vector was then transfected into BOSC23 cells which as reported in Pear, *et al., Proc. Natl. Acad. Sci. (USA), 90*: 8392-8396 (1993), incorporated by reference herein. The supernatant was then collected 48 hours later and used to infect NIH3T3 cells. The infected cells were selected by growth for 2 weeks in puromycin. COS cells were transfected with 10 μg of the pMT2 vector, which is reported in Kaufman, *et al, Cell. Biol., 9*: 946-958, incorporated by reference herein, containing the *BMX* cDNA insert using the calcium phosphate method. After 36-48 hours of growth, the cells were extracted with the electrophoresis sample buffer 2.5 % sodium dodecyl sulfate, 0.125 M Tris-HCl, pH 6.8 for Western blotting or with ice-cold RIPA buffer (50mM TrisHCl pH 7.5, NaCl 150 mM, 1% Triton X100, 1% sodium deoxycholate, 0.1% SDS, containing 10 mg/ml pepstatin, 100 5g/ml leupeptin, 0.05 TIU/ml aprotinin, 1 mM PMSF and 1 mM activated sodium orthovanadate) for immunoprecipitation. The clarified supernatants were immunoprecipitated with 5 μl of anti-*BMX* antiserum raised in rabbits using a GST-fusion protein (Pharmacia) engineered to express *BMX* amino acid residues 599-675. Preimmune serum and anti-SEX antiserum against an unrelated protein (L.T., in preparation) were used as controls.

Samples were analyzed in 7.5% SDS-PAGE followed by Western blotting and detection using a

1: 1000 dilution of the anti-*BMX* antiserum (Figure 2B) or the PY20 anti-phosphotyrosine monoclonal antibodies as shown in Figure 2C (Zymed), followed by peroxidase-conjugated antibodies against mouse immunoglobulins and ECL detection according to the manufacturer's instructions (Amersham). Alternatively, the immunoprecipitates were subjected to a kinase reaction in 25 mM HEPES, pH 7.2, 100mM NaCl, 5 mM $MgCl_2$, 10 mM $MnCl_2$ and 10 mCi [^{32}P]-ATP for 10 minutes at room temperature, followed by SDS-PAGE and autoradiography.

Results are shown in Figures 2A-2D. Figure 2A shows Northern blotting analysis of RNA from human umbilical vein endothelial cells using the *BMX* probe (labelled *BMX* NB in the Figure). A 2.7 kb mRNA band is seen. Immunoprecipitation of the *BMX* protein followed by immunoblotting with anti-*BMX* antibodies resulted in the detection of a weak band of 80 kD apparent molecular weight as shown in Figure 2B and 3. That band was not seen in control immunoprecipitates. Immunoprecipitation and immunoblotting of *BMX* from NIH3T3 cells expressing a *BMX* retrovirus and from COS cells transfected with a *BMX* plasmid expression vector also resulted in the detection of a 80 kD polypeptide, which was tyrosyl phosphorylated as shown in Figure 2C (α-PTyr WB). However, that polypeptide was only weakly labelled in immunocomplex kinase reactions [^{32}P]-ATP, as shown in Figure 2D.

EXAMPLE 3

Chromosomal Localization of the *BMX* Gene

A Southern blot made from 24 interspecies somatic cell hybrids was obtained from the Mutant

Cell Repository of the Coriell Institute (Camden, NJ).

In order to determine the chromosomal localization of the *BMX* gene, DNAs from human rodent somatic cell hybrids containing defined sets of human chromosomes were analyzed by Southern blotting and hybridization with the *BMX* probe. Among 24 DNA samples, human-specific signals were observed in two human/Chinese hamster hybrids, one containing human chromosomes 1 and X and the other only the human X chromosome. This analysis indicated that the *BMX* gene is located in chromosome X. Thus the name Bone Marrow kinase gene on the X chromosome, *BMX*, was chosen. A human placenta cosmid library in pWE15, described in Lichter, *et al., Human Genet., 80*: 224-234 (1988), and a human X-chromosome yeast artificial chromosome (YAC) library were screened with the [^{32}P]-labelled insert of the *BMX* cDNA. Positive clones were rescreened until pure, and verified by Southern blotting and hybridization.

Slides with human metaphase chromosomes, prepared from 5-bromo deoxyuridine-synchronized lymphocyte cultures were prehybridized and hybridized essentially as described in Lichter, *et al., Human Genet., 80*: 224-234 (1988), incorporated by reference herein. Four *Eco*RI fragments (1, 1.5, 2.3 and 2.5 kb) of the *BMX* cosmid were used as probes after labeling with biotin-16-dUTP by nick translation. The four probes were pooled in a mixture of 50% formamide, 2xSSC, 1% Tween 20, 10% dextran sulfate, 25μg Cot-I DNA and 8 mg salmon sperm DNA, denatured at 75 °C for 5 minutes and then preannealed at 37 °C for 30 minutes. After hybridization, the slides were stringently washed,

the signal was made fluorescent, amplified, and the chromosomes were counterstained with propidiumiodide and DAPI. The results were analyzed and photographed in a confocal laser scanning microscope (Zeiss).

A genomic cosmid clone isolated by hybridization with the *BMX* cDNA gave signals from chromosomes X and 18 in the hybrid panel. Therefore, all four *BMX*-positive *Eco*RI fragments of this clone were labelled and used inflorescence *in situ* hybridization to further localize the *BMX* gene. This probe gave a specific signal in Xp22.2-p21 (Fig. 4). The *BMX* cDNA was then hybridized to YACs from the Xp21 and Xp22 region. The 400 kb ICRF YAC 900G1096 and the 350 kb CEPH YAC244G7 were positive for *BMX*. Both of these YACs were non-chimeric as analyzed by FISH; the former was positive for the DXS207 and DXS197 loci and the latter for only DXS197. As *BMX* was negative on YACs positive for DXS197 and DXS43, this maps *BMX* between the DXS197 and DXS207 loci in band Xp22.2.

The invention has been described in terms of its preferred embodiments. Accordingly, additional aspects of the invention will be apparent to the ordinarily-skilled artisan upon reading the present application.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Helsinki University Licensing Ltd Oy

(ii) TITLE OF INVENTION: Cytoplasmic Tyrosine Kinase

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: OY JALO ANT-WUORINEN AB
(B) STREET: Iso Roobertinkatu 4-6 A
(C) CITY: Helsinki
(E) COUNTRY: Finland
(F) ZIP: FIN-00120

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Karvinen, Leena
(C) REFERENCE/DOCKET NUMBER: 28218

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +358 0 648606
(B) TELEFAX: +358 0 640575
(C) TELEX: 123505 JALO FI

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTCTAGAAR AARTTYGTSC ACMGRGAC 28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTCTAGARG GCCATCCAYT TVACHGG 27

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Asp Thr Lys Ser Ile Leu Glu Glu Leu Leu Leu Lys Arg Ser Gln
1               5                   10                  15

Gln Lys Lys Lys Met Ser Pro Asn Asn Tyr Lys Glu Arg Leu Phe Val
            20                  25                  30

Leu Thr Lys Thr Asn Leu Ser Tyr Tyr Glu Tyr Asp Lys Met Lys Arg
        35                  40                  45

Gly Ser Arg Lys Gly Ser Ile Glu Ile Lys Lys Ile Arg Cys Val Glu
    50                  55                  60

Lys Val Asn Leu Glu Glu Gln Thr Pro Val Glu Arg Gln Tyr Pro Phe
65                  70                  75                  80

Gln Ile Val Tyr Lys Asp Gly Leu Leu Tyr Val Tyr Ala Ser Asn Glu
                85                  90                  95

Glu Ser Arg Ser Gln Trp Leu Lys Ala Leu Gln Lys Glu Ile Arg Gly
            100                 105                 110

Asn Pro His Leu Leu Val Lys Tyr His Ser Gly Phe Phe Val Asp Gly
        115                 120                 125

Lys Phe Leu Cys Cys Gln Gln Ser Cys Lys Ala Ala Pro Gly Cys Thr
    130                 135                 140

Leu Trp Glu Ala Tyr Ala Asn Leu His Thr Ala Val Asn Glu Glu Lys
145                 150                 155                 160

His Arg Val Pro Thr Phe Pro Asp Arg Val Leu Lys Ile Pro Arg Ala
                165                 170                 175

Val Pro Val Leu Lys Met Asp Ala Pro Ser Ser Ser Thr Thr Leu Ala
            180                 185                 190

Gln Tyr Asp Asn Glu Ser Lys Lys Asn Tyr Gly Ser Gln Pro Pro Ser
        195                 200                 205

Ser Ser Thr Ser Leu Ala Gln Tyr Asp Ser Asn Ser Lys Lys Ile Tyr
    210                 215                 220

Gly Ser Gln Pro Asn Phe Asn Met Gln Tyr Ile Pro Arg Glu Asp Phe
225                 230                 235                 240

Pro Asp Trp Trp Gln Val Arg Lys Leu Lys Ser Ser Ser Ser Ser Glu
                245                 250                 255
```

```
Asp Val Ala Ser Ser Asn Gln Lys Glu Arg Asn Val Asn His Thr Thr
            260                 265                 270

Ser Lys Ile Ser Trp Glu Phe Pro Glu Ser Ser Ser Ser Glu Glu Glu
        275                 280                 285

Glu Asn Leu Asp Asp Tyr Asp Trp Phe Ala Gly Asn Ile Ser Arg Ser
    290                 295                 300

Gln Ser Glu Gln Leu Leu Arg Gln Lys Gly Lys Glu Gly Ala Phe Met
305                 310                 315                 320

Val Arg Asn Ser Ser Gln Val Gly Met Tyr Thr Val Ser Leu Phe Ser
                325                 330                 335

Lys Ala Val Asn Asp Lys Lys Gly Thr Val Lys His Tyr His Val His
            340                 345                 350

Thr Asn Ala Glu Asn Lys Leu Tyr Leu Ala Glu Asn Tyr Cys Phe Asp
        355                 360                 365

Ser Ile Pro Lys Leu Ile His Tyr His Gln His Asn Ser Ala Gly Met
    370                 375                 380

Ile Thr Arg Leu Arg His Pro Val Ser Thr Lys Ala Asn Lys Val Pro
385                 390                 395                 400

Asp Ser Val Ser Leu Gly Asn Gly Ile Trp Glu Leu Lys Arg Glu Glu
                405                 410                 415

Ile Thr Leu Leu Lys Glu Leu Gly Ser Gly Gln Phe Gly Val Val Gln
            420                 425                 430

Leu Gly Lys Trp Lys Gly Gln Tyr Asp Val Ala Val Lys Met Ile Lys
        435                 440                 445

Glu Gly Ser Met Ser Glu Asp Glu Phe Phe Gln Glu Ala Gln Thr Met
    450                 455                 460

Met Lys Leu Ser His Pro Lys Leu Val Lys Phe Tyr Gly Val Cys Ser
465                 470                 475                 480

Lys Glu Tyr Pro Ile Tyr Ile Val Thr Glu Tyr Ile Ser Asn Gly Cys
                485                 490                 495

Leu Leu Asn Tyr Leu Arg Ser His Gly Lys Gly Leu Glu Pro Ser Gln
            500                 505                 510

Leu Leu Glu Met Cys Tyr Asp Val Cys Glu Gly Met Ala Phe Leu Glu
        515                 520                 525

Ser His Gln Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val
    530                 535                 540

Asp Arg Asp Leu Cys Val Lys Val Ser Asp Phe Gly Met Thr Arg Tyr
545                 550                 555                 560

Val Leu Asp Asp Gln Tyr Val Ser Ser Val Gly Thr Lys Phe Pro Val
                565                 570                 575

Lys Trp Ser Ala Pro Glu Val Phe His Tyr Phe Lys Tyr Ser Ser Lys
            580                 585                 590

Ser Asp Val Trp Ala Phe Gly Ile Leu Met Trp Glu Val Phe Ser Leu
        595                 600                 605
```

```
Gly Lys Gln Pro Tyr Asp Leu Tyr Asp Asn Ser Gln Val Val Leu Lys
    610                 615                 620

Val Ser Gln Gly His Arg Leu Tyr Arg Pro His Leu Ala Ser Asp Thr
625                 630                 635                 640

Ile Tyr Gln Ile Met Tyr Ser Cys Trp His Glu Leu Pro Glu Lys Arg
                645                 650                 655

Pro Thr Phe Gln Gln Leu Leu Ser Ser Ile Glu Pro Leu Arg Glu Lys
            660                 665                 670

Asp Lys His
        675
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 659 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Ala Val Ile Leu Glu Ser Ile Phe Leu Lys Arg Ser Gln Gln
1               5                   10                  15

Lys Lys Lys Thr Ser Pro Leu Asn Phe Lys Lys Arg Leu Phe Leu Leu
            20                  25                  30

Thr Val His Lys Leu Ser Tyr Tyr Glu Tyr Asp Phe Glu Arg Gly Arg
        35                  40                  45

Arg Gly Ser Lys Lys Gly Ser Ile Asp Val Glu Lys Ile Thr Cys Val
    50                  55                  60

Glu Thr Val Val Pro Glu Lys Asn Pro Pro Pro Glu Arg Gln Ile Pro
65                  70                  75                  80

Arg Arg Gly Glu Glu Ser Ser Glu Met Glu Gln Ile Ser Ile Ile Glu
                85                  90                  95

Arg Phe Pro Tyr Pro Phe Gln Val Val Tyr Asp Glu Gly Pro Leu Tyr
            100                 105                 110

Val Phe Ser Pro Thr Glu Glu Leu Arg Lys Arg Trp Ile His Gln Leu
        115                 120                 125

Lys Asn Val Ile Arg Tyr Asn Ser Asp Leu Val Gln Lys Tyr His Pro
    130                 135                 140

Cys Phe Trp Ile Asp Gly Gln Tyr Leu Cys Cys Ser Gln Thr Ala Lys
145                 150                 155                 160

Asn Ala Met Gly Cys Gln Ile Leu Glu Asn Arg Asn Gly Ser Leu Lys
                165                 170                 175

Pro Gly Ser Ser His Arg Lys Thr Lys Lys Pro Leu Pro Pro Thr Pro
            180                 185                 190
```

Glu Glu Asp Gln Ile Leu Lys Lys Pro Leu Pro Pro Glu Pro Ala Ala
195 200 205

Ala Pro Val Ser Thr Ser Glu Leu Lys Lys Val Val Ala Leu Tyr Asp
210 215 220

Tyr Met Pro Met Asn Ala Asn Asp Leu Gln Leu Arg Lys Gly Asp Glu
225 230 235 240

Tyr Phe Ile Leu Glu Glu Ser Asn Leu Pro Trp Trp Arg Ala Arg Asp
245 250 255

Lys Asn Gly Gln Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Glu Ala
260 265 270

Glu Asp Ser Ile Glu Met Tyr Glu Trp Tyr Ser Lys His Met Thr Arg
275 280 285

Ser Gln Ala Glu Gln Leu Leu Lys Gln Glu Gly Lys Glu Gly Gly Phe
290 295 300

Ile Val Arg Asp Ser Ser Lys Ala Gly Lys Tyr Thr Val Ser Val Phe
305 310 315 320

Ala Lys Ser Thr Gly Asp Pro Gln Gly Val Ile Arg His Tyr Val Val
325 330 335

Cys Ser Thr Pro Gln Ser Gln Tyr Tyr Leu Ala Glu Lys His Leu Phe
340 345 350

Ser Thr Ile Pro Glu Leu Ile Asn Tyr His Gln His Asn Ser Ala Gly
355 360 365

Leu Ile Ser Arg Leu Lys Tyr Pro Val Ser Gln Gln Asn Lys Asn Ala
370 375 380

Pro Ser Thr Ala Gly Leu Gly Tyr Gly Ser Trp Glu Ile Asp Pro Lys
385 390 395 400

Asp Leu Thr Phe Leu Lys Glu Leu Gly Thr Gly Gln Phe Gly Val Val
405 410 415

Lys Tyr Gly Lys Trp Arg Gly Gln Tyr Asp Val Ala Ile Lys Met Ile
420 425 430

Lys Glu Gly Ser Met Ser Glu Asp Glu Phe Ile Glu Glu Ala Lys Val
435 440 445

Met Met Asn Leu Ser His Glu Lys Leu Val Gln Leu Tyr Gly Val Cys
450 455 460

Thr Lys Gln Arg Pro Ile Phe Ile Ile Thr Glu Tyr Met Ala Asn Gly
465 470 475 480

Cys Leu Leu Asn Tyr Leu Arg Glu Met Arg His Arg Phe Gln Thr Gln
485 490 495

Gln Leu Leu Glu Met Cys Lys Asp Val Cys Glu Ala Met Glu Tyr Leu
500 505 510

Glu Ser Lys Gln Phe Leu His Arg Asp Leu Ala Ala Arg Asn Cys Leu
515 520 525

Val Asn Asp Gln Gly Val Val Lys Val Ser Asp Phe Gly Leu Ser Arg
530 535 540

```
Tyr Val Leu Asp Asp Glu Tyr Thr Ser Ser Val Gly Ser Lys Phe Pro
545                 550                 555                 560

Val Arg Trp Ser Pro Pro Glu Val Leu Met Tyr Ser Lys Phe Ser Ser
                565                 570                 575

Lys Ser Asp Ile Trp Ala Phe Gly Val Leu Met Trp Glu Ile Tyr Ser
            580                 585                 590

Leu Gly Lys Met Pro Tyr Glu Arg Phe Thr Asn Ser Glu Thr Ala Glu
        595                 600                 605

His Ile Ala Gln Gly Leu Arg Leu Tyr Arg Pro His Leu Ala Ser Glu
    610                 615                 620

Lys Val Tyr Thr Ile Met Tyr Ser Cys Trp His Glu Lys Ala Asp Glu
625                 630                 635                 640

Arg Pro Thr Phe Lys Ile Leu Leu Ser Asn Ile Leu Asp Val Met Asp
                645                 650                 655

Glu Glu Ser
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 620 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asn Asn Phe Ile Leu Leu Glu Glu Gln Leu Ile Lys Lys Ser Gln
1               5                   10                  15

Gln Lys Arg Arg Thr Ser Pro Ser Asn Phe Lys Val Arg Phe Phe Val
            20                  25                  30

Leu Thr Lys Ala Ser Leu Ala Tyr Phe Glu Asp Arg His Gly Lys Lys
        35                  40                  45

Arg Thr Leu Lys Gly Ser Ile Glu Leu Ser Arg Ile Lys Cys Val Glu
    50                  55                  60

Ile Val Lys Ser Asp Ile Ser Ile Pro Cys His Tyr Lys Tyr Pro Phe
65                  70                  75                  80

Gln Val Val His Asp Asn Tyr Leu Leu Tyr Val Phe Ala Pro Asp Arg
                85                  90                  95

Glu Ser Arg Gln Arg Trp Val Leu Ala Leu Lys Glu Glu Thr Arg Asn
            100                 105                 110

Asn Asn Ser Leu Val Pro Lys Tyr His Pro Asn Phe Trp Met Asp Gly
        115                 120                 125

Lys Trp Arg Cys Cys Ser Gln Leu Glu Lys Leu Ala Thr Gly Cys Ala
    130                 135                 140
```

Gln Tyr Asp Pro Thr Lys Asn Ala Ser Lys Lys Pro Leu Pro Pro Thr
145 150 155 160

Pro Glu Asp Asn Arg Arg Pro Leu Trp Glu Pro Glu Glu Thr Val Val
165 170 175

Ile Ala Leu Tyr Asp Tyr Gln Thr Asn Asp Pro Gln Glu Leu Ala Leu
180 185 190

Arg Arg Asn Glu Glu Tyr Cys Leu Leu Asp Ser Ser Glu Ile His Trp
195 200 205

Trp Arg Val Gln Asp Arg Asn Gly His Glu Gly Tyr Val Pro Ser Ser
210 215 220

Tyr Leu Val Glu Lys Ser Pro Asn Asn Leu Glu Thr Tyr Glu Trp Tyr
225 230 235 240

Asn Lys Ser Ile Ser Arg Asp Lys Ala Glu Lys Leu Leu Leu Asp Thr
245 250 255

Gly Lys Glu Gly Ala Phe Met Val Arg Asp Ser Arg Thr Ala Gly Thr
260 265 270

Tyr Thr Val Ser Val Phe Thr Lys Ala Val Val Ser Glu Asn Asn Pro
275 280 285

Cys Ile Lys His Tyr His Ile Lys Glu Thr Asn Asp Asn Pro Lys Arg
290 295 300

Tyr Tyr Val Ala Glu Lys Tyr Val Phe Asp Ser Ile Pro Leu Leu Ile
305 310 315 320

Asn Tyr His Gln His Asn Gly Gly Gly Leu Val Thr Arg Leu Arg Tyr
325 330 335

Pro Val Cys Phe Gly Arg Gln Lys Ala Pro Val Thr Ala Gly Leu Arg
340 345 350

Tyr Gly Lys Trp Val Ile Asp Pro Ser Glu Leu Thr Phe Val Gln Glu
355 360 365

Ile Gly Ser Gly Gln Phe Gly Leu Val His Leu Gly Tyr Trp Leu Asn
370 375 380

Lys Asp Lys Val Ala Ile Lys Thr Ile Arg Glu Gly Ala Met Ser Glu
385 390 395 400

Glu Asp Phe Ile Glu Glu Ala Glu Val Met Met Lys Leu Ser His Pro
405 410 415

Lys Leu Val Gln Leu Tyr Gly Val Cys Leu Glu Gln Ala Pro Ile Cys
420 425 430

Leu Val Phe Glu Phe Met Glu His Gly Cys Leu Ser Asp Tyr Leu Arg
435 440 445

Thr Gln Arg Gly Leu Phe Ala Ala Glu Thr Leu Leu Gly Met Cys Leu
450 455 460

Asp Val Cys Glu Gly Met Ala Tyr Leu Glu Glu Ala Cys Val Ile His
465 470 475 480

Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Gly Glu Asn Gln Val Ile
485 490 495

```
Lys Val Ser Asp Phe Gly Met Thr Arg Phe Val Leu Asp Asp Gln Tyr
            500                 505                 510

Thr Ser Ser Thr Gly Thr Lys Phe Pro Val Lys Trp Ala Ser Pro Glu
        515                 520                 525

Val Phe Ser Phe Ser Arg Tyr Ser Ser Lys Ser Asp Val Trp Ser Phe
    530                 535                 540

Gly Val Leu Met Trp Glu Val Phe Ser Glu Gly Lys Ile Pro Tyr Glu
545                 550                 555                 560

Asn Arg Ser Asn Ser Glu Val Val Glu Asp Ile Ser Thr Gly Phe Arg
                565                 570                 575

Leu Tyr Lys Pro Arg Leu Ala Ser Thr His Val Tyr Gln Ile Met Asn
            580                 585                 590

His Cys Trp Lys Glu Arg Pro Glu Asp Arg Pro Ala Phe Ser Arg Leu
        595                 600                 605

Leu Arg Gln Leu Ala Glu Ile Ala Glu Ser Gly Leu
    610                 615                 620
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 630 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Asn Phe Asn Thr Ile Leu Glu Glu Ile Leu Ile Lys Arg Ser Gln
1               5                   10                  15

Gln Lys Lys Lys Thr Ser Leu Leu Asn Tyr Lys Glu Arg Leu Cys Val
            20                  25                  30

Leu Pro Lys Ser Val Leu Ser Tyr Tyr Glu Gly Arg Ala Glu Lys Lys
        35                  40                  45

Tyr Arg Lys Gly Val Ile Asp Ile Ser Lys Ile Lys Cys Val Glu Ile
    50                  55                  60

Val Lys Asn Asp Asp Gly Val Ile Pro Cys Gln Asn Lys Phe Pro Phe
65                  70                  75                  80

Gln Val Val His Asp Ala Asn Thr Leu Tyr Ile Phe Ala Pro Ser Pro
                85                  90                  95

Gln Ser Arg Asp Arg Trp Val Lys Lys Leu Lys Glu Glu Ile Lys Asn
            100                 105                 110

Asn Asn Asn Ile Met Ile Lys Tyr His Pro Lys Phe Trp Ala Asp Gly
        115                 120                 125

Ser Tyr Gln Cys Cys Arg Gln Thr Glu Lys Leu Ala Pro Gly Cys Glu
    130                 135                 140
```

Lys Tyr Asn Leu Phe Glu Ser Ser Ile Arg Lys Thr Leu Pro Pro Ala
145 150 155 160

Pro Glu Ile Lys Lys Arg Arg Pro Pro Pro Pro Ile Pro Pro Glu Lys
165 170 175

Lys Asn Thr Glu Glu Ile Val Val Ala Met Tyr Asp Phe Gln Ala Thr
180 185 190

Glu Ala His Asp Leu Arg Leu Glu Arg Gly Gln Glu Tyr Ile Ile Leu
195 200 205

Glu Lys Asn Asp Leu His Trp Trp Arg Ala Arg Asp Lys Tyr Gly Ser
210 215 220

Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Gly Lys Lys Ser Asn Asn
225 230 235 240

Leu Asp Gln Tyr Glu Trp Tyr Cys Arg Asn Thr Asn Arg Ser Lys Ala
245 250 255

Glu Gln Leu Leu Arg Thr Glu Asp Lys Glu Gly Gly Phe Met Val Arg
260 265 270

Asp Ser Ser Gln Pro Gly Leu Tyr Thr Val Ser Leu Tyr Thr Lys Phe
275 280 285

Gly Gly Glu Gly Ser Ser Gly Phe Arg His Tyr His Ile Lys Glu Thr
290 295 300

Ala Thr Ser Pro Lys Lys Tyr Tyr Leu Ala Glu Lys His Ala Phe Gly
305 310 315 320

Ser Ile Pro Glu Ile Ile Glu Tyr His Lys His Asn Ala Ala Gly Leu
325 330 335

Val Thr Arg Leu Arg Tyr Pro Val Ser Thr Lys Gly Lys Asn Ala Pro
340 345 350

Thr Thr Ala Gly Phe Ser Tyr Asp Lys Trp Glu Ile Asn Pro Ser Glu
355 360 365

Leu Thr Phe Met Arg Glu Leu Gly Ser Gly Leu Phe Gly Val Val Arg
370 375 380

Leu Gly Lys Trp Arg Ala Gln Tyr Lys Val Ala Ile Lys Ala Ile Arg
385 390 395 400

Glu Gly Ala Met Cys Glu Glu Asp Phe Ile Glu Glu Ala Lys Val Met
405 410 415

Met Lys Leu Thr His Pro Lys Leu Val Gln Leu Tyr Gly Val Cys Thr
420 425 430

Gln Gln Lys Pro Ile Tyr Ile Val Thr Glu Phe Met Glu Arg Gly Cys
435 440 445

Leu Leu Asn Phe Leu Arg Gln Arg Gln Gly His Phe Ser Arg Asp Met
450 455 460

Leu Leu Ser Met Cys Gln Asp Val Cys Glu Gly Met Glu Tyr Leu Glu
465 470 475 480

Arg Asn Ser Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val
485 490 495

```
Asn Glu Ala Gly Val Val Lys Val Ser Asp Phe Gly Met Ala Arg Tyr
            500                 505                 510

Val Leu Asp Asp Gln Tyr Thr Ser Ser Ser Gly Ala Lys Phe Pro Val
        515                 520                 525

Lys Trp Cys Pro Pro Glu Val Phe Asn Tyr Ser Arg Gly Ser Ser Lys
    530                 535                 540

Ser Asp Val Trp Ser Phe Gly Val Leu Met Trp Glu Ile Phe Thr Glu
545                 550                 555                     560

Gly Arg Met Pro Phe Glu Lys Asn Thr Asn Tyr Glu Val Val Thr Met
                565                 570                 575

Val Thr Arg Gly His Arg Leu His Arg Pro Lys Leu Ala Thr Lys Tyr
            580                 585                 590

Leu Tyr Glu Val Met Leu Arg Cys Trp Gln Glu Arg Pro Glu Gly Arg
        595                 600                 605

Pro Ser Phe Glu Asp Leu Leu Arg Thr Ile Asp Glu Leu Val Glu Cys
    610                 615                 620

Glu Glu Thr Phe Gly Arg
625                 630
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Val Ile Lys Glu Gly Leu Lys Lys Trp Lys Arg Phe Val Leu Leu Ser
1               5                   10                  15

Tyr Tyr Lys Gly Leu Ile Asp Leu Ile Ile Val Glu Phe Ile Val Leu
            20                  25                  30

Ile Leu Ala Glu Glu Glu Arg Trp Val Ala Leu Ile Ala Leu Tyr Asp
        35                  40                  45

Tyr Asp Leu Leu Gly Asp Ile Leu Trp Trp Gly Pro Tyr Val Trp Ile
    50                  55                  60

Ser Arg Ala Leu Leu Gly Phe Leu Val Arg Gly Tyr Ser Val His Tyr
65                  70                  75                  80

Phe Leu Ile Pro Val
                85
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Val Ala Leu Tyr Leu Gly Lys Ala Ile Glu Gly Gly Asp Leu Ser
1 5 10 15

Val Gly Glu Lys Asn Ala Glu Tyr Glu Val Ile Asp Asp Ser Gln Glu
20 25 30

His Trp Trp Lys Val Lys Asp Ala Leu Gly Asn Val Gly Tyr Ile Pro
35 40 45

Ser Asn Tyr Val Gln Ala Glu Ala Leu Leu Gly Leu Glu Arg Tyr Glu
50 55 60

Trp Tyr Val Gly Tyr Met Ser Arg Gln Arg Ala Glu Ser Leu Leu Lys
65 70 75 80

Gln Gly Asp Lys Glu Gly Cys Phe Val Val Arg Lys Ser Ser Thr Lys
85 90 95

Gly Leu Tyr Thr Leu Ser Leu His Thr Lys Val Pro Gln Ser His Val
100 105 110

Lys His Tyr His Ile Lys Gln Asn Ala Arg Cys Glu Tyr Tyr Leu Ser
115 120 125

Glu Lys His Cys Cys Glu Thr Ile Pro Asp Leu Ile Asn Tyr His Arg
130 135 140

His Asn Ser Ala Gly Leu Ala Cys Arg Leu Lys Ser Ser Pro Cys Asp
145 150 155 160

Arg Pro Val Pro Pro Thr Ala Gly Leu Ser His Asp Lys Trp Glu Ile
165 170 175

His Pro Ile Gln Leu Met Leu Met Glu Glu Leu Gly Ser Gly Gln Phe
180 185 190

Gly Val Val Arg Arg Gly Lys Trp Arg Gly Ser Ile Asp Thr Ala Val
195 200 205

Lys Met Met Lys Glu Gly Thr Met Ser Glu Asp Asp Phe Ile Glu Glu
210 215 220

Ala Lys Val Met Thr Lys Leu Gln His Pro Asn Leu Val Gln Leu Tyr
225 230 235 240

Gly Val Cys Thr Lys His Arg Pro Ile Tyr Ile Val Thr Glu Tyr Met
245 250 255

Lys His Gly Ser Leu Leu Asn Tyr Leu Arg Arg His Glu Lys Thr Leu
260 265 270

Ile Gly Asn Met Gly Leu Leu Leu Asp Met Cys Ile Gln Val Ser Lys
275 280 285

Gly Met Thr Tyr Leu Glu Arg His Asn Tyr Ile His Arg Asp Leu Ala
290 295 300

Ala Arg Asn Cys Leu Val Gly Ser Glu Asn Val Val Lys Val Ala Asp
305 310 315 320

```
Phe Gly Leu Ala Arg Tyr Val Leu Asp Asp Gln Tyr Thr Ser Ser Gly
                325                 330                 335

Gly Thr Lys Phe Pro Ile Lys Trp Ala Pro Pro Glu Val Leu Asn Tyr
            340                 345                 350

Thr Arg Phe Ser Ser Lys Ser Asp Val Trp Ala Tyr Gly Val Leu Met
        355                 360                 365

Trp Glu Ile Phe Thr Cys Gly Lys Met Pro Tyr Gly Arg Leu Lys Asn
    370                 375                 380

Thr Glu Val Val Glu Arg Val Gln Arg Gly Ile Ile Leu Glu Lys Pro
385                 390                 395                 400

Lys Ser Cys Ala Lys Glu Ile Tyr Asp Val Met Lys Leu Cys Trp Ser
                405                 410                 415

His Gly Pro Glu Glu Arg Pro Ala Phe Arg Val Leu Met Asp Gln Leu
            420                 425                 430

Ala Leu Val Ala Gln Thr Leu Thr Asp
        435                 440
```

CLAIMS

What is claimed is:

1. A protein comprising the amino acid sequence shown in SEQ ID NO: 3.

2. A fragment of the protein according to claim 1 which is capable of stimulating growth of hematopoietic cells.

3. A DNA encoding the protein according to claim 1.

4. A DNA encoding the protein fragment according to claim 2.

5. A method for detecting growth of hematopoietic cells, comprising the steps of

(a) exposing tissue comprising said hematopoietic cells to a detectably labelled DNA according to claim 3;

(b) washing said tissue; and

(c) detecting said label in said tissue.

6. An antibody which is specifically reactive with the protein according to claim 1.

```
....Bmx   MDTKSILEEL  LLKRSQQKKK  MSPNNYKERL  FVLTKTNLSY  YEYD--KM
....Btk   M.AAVILESI  FLKRSQQKKK  TSPLNFKKRL  FLLTVHKLSY  YEYDFERG
....Emt   MNNFILLEEQ  LIKKSQQKRR  TSPSNFKVRF  FVLTKASLAY  FED--RHG
....Tec   MNFNTILEEI  LIKRSQQKKK  TSLLNYKERL  CVLPKSVLSY  YEG---RA
consens   ....vikeg.  l.kk......  .....wk.r.  fvl....lsy  y.......

....Bmx   ----------  YPFQIVYKDG  LLYVYASNEE  SRSQWLKALQ  KEIRGNPH
....Btk   EQISIIERFP  YPFQVVYDEG  PLYVFSPTEE  LRKRWIHQLK  NVIRYNSD
....Emt   SDISIPCHYK  YPFQVVHDNY  LLYVFAPDRE  SRQRWVLALK  EETRNNNS
....Tec   DDGVIPCQNK  FPFQVVHDAN  TLYIFAPSPQ  SRDRWVKKLK  EEIKNNNN
consens   ..........  ..f.iv....  .lil.a..ee  er..wv.al.  ..i.....

....Bmx   EEKHRVPTFP  DRVLKIPRAV  PVLKMDAPSS  STTLAQYDNE  SKKNYGSQ
....Btk   SHRKTKKPLP  PTPEEDQILK  KPLPPEPAAA  PVSTSELKK-  --------
....Emt   ----SKKPLP  PTPED---NR  R-----PLWE  PEET------  --------
....Tec   ----IRKTLP  PAPEI----K  KRRPPPPIPP  EKKNTEEI--  --------
DSrc28C   (./.)-----  ----------  ----------  ----------  --------
consens   ..........  ..........  ..........  ..........  ........

....Bmx   VRKLKSSSSS  EDVASSNQKE  RNVNHTTSKI  SWEFPESSSS  EEEENLDD
....Btk   ARDKNGQEGY  IPSNYVTEA.  ----------  ----------  --EDSIEM
....Emt   VQDRNGHEGY  VPSSYLVEKS  ----------  ----------  --PNNLET
....Tec   ARDKYGSEGY  IPSNYVTGK-  ----------  ----------  -KSNNLDQ
DSrc28C   VKDALGNVGY  IPSNYVQAE-  ----------  ----------  -ALLGLER
consens   ........g.  .p..yv....  ..........  ..........  ........

....Bmx   SKA-VNDKKG  TVKHYHVHTN  AEN--KLYLA  ENYCFDSIPK  LIHYHQHN
```

Figure 1
(1/4)

```
KR GSRKGSIEIK KIRCVEKVNL EEQTPVERQ- ---------- 77
RR GSKKGSIDVE KITCVETVVP EKNPPPERQI PRRGEESSEM 89    PH
KK RTLKGSIELS RIKCVEIVK- ---------- ---------- 67
EK KYRKGVIDIS KIKCVEIVKN ---------- ---------- 67
.. ...kglidl. .i.ive.... .......... .......... ..

LL VKYHSGFFVD GKFLCCQQSC KAAPGCTLWE AYANLHTAVN 157
LV QKYHPCFWID GQYLCCSQTA KNAMGCQILE NRNGSLKPGS 179
LV PKYHPNFWMD GKWRCCSQLE KLATGCAQYD PTKNA----- 152
IM IKYHPKFWAD GSYQCCRQTE KLAPGCEKYN LFESS----- 157
.. .......... .......... .......... .......... ...

PP SSSTSLAQYD SNSKKIYGSQ -PNFNMQYIP RED-FPDWWQ 245
-- ----VVALYD YMPMNANDLQ -LRKGDEYFI LEESNLPWWR 253
-- ---VVIALYD YQTNDPQELA -LRRNEEYCL LDSSEIHWWR 210    SH3
-- ----VVAMYD FQATEAHDLR -LERGQEYII LEKNDLHWWR 217
-- ----VVALYL GKAIEGGDLS VGEKNAEYEV IDDSQEHWWK 185
.. ......alyd y......dl. .l..gd...i l......ww. ...

YD WFAGNISRSQ SEQLLRQKGK EGAFMVRNSS QVGMYTVSLF 335
YE WYSKHMTRSQ AEQLLKQEGK EGGFIVRDSS KAGKYTVSVF 320
YE WYNKSISRDK AEKLLLDTGK EGAFMVRDSR TAGTYTVSVF 278    SH2
YE WYCRNTNRSK AEQLLRTEDK EGGFMVRDSS QPGLYTVSLY 275
YE WYVGYMSRQR AESLLKQGDK EGCFVVRKSS TKGLYTLSLH 253
.. w....isr.. a..ll..... .g.flvr... ..g.y..s.. ...

SA GMITRLRHPV STK-ANKVPD SVSLGNGIWE LKREEITLLK 421
```

Figure 1
(2/4)

```
....Btk    AKS-TGDPQG  VIRHYVVCST  PQS--QYYLA  EKHLFSTIPE  LINYHQHN
....Emt    TKAVVSENNP  CIKHYHIKET  NDNPKRYYVA  EKYVFDSIPL  LINYHQHN
....Tec    TKF-GGEGSS  GFRHYHIKET  ATSPKKYYLA  EKHAFGSIPE  IIEYHKHN
DSrc28C    TKV----PQS  HVKHYHIKQN  A--RCEYYLS  EKHCCETIPD  LINYHRHN
consens    ..........  .v.hy.....  ..........  ....f.....  li......

                 ATP binding
....Bmx    ELGSGQFGVV  QLGKWKGQYD  VAVKMIKEGS  MSEDEFFQEA  QTMMKLSH
....Btk    ELGTGQFGVV  KYGKWRGQYD  VAIKMIKEGS  MSEDEFIEEA  KVMMNLSH
....Emt    EIGSGQFGLV  HLGYWLNKDK  VAIKTIREGA  MSEEDFIEEA  EVMMKLSH
....Tec    ELGSGLFGVV  RLGKWRAQYK  VAIKAIREGA  MCEEDFIEEA  KVMMKLTH
DSrc28C    ELGSGQFGVV  RRGKWRGSID  TAVKMMKEGT  MSEDDFIEEA  KVMTKLQH

....Bmx    -SQLLEMCYD  VCEGMAFLES  HQFIHRDLAA  RNCLVDRDLC  VKVSDFGM
....Btk    -QQLLEMCKD  VCEAMEYLES  KQFLHRDLAA  RNCLVNDQGV  VKVSDFGL
....Emt    -ETLLGMCLD  VCEGMAYLEE  ACVIHRDLAA  RNCLVGENQV  IKVSDFGM
....Tec    -DMLLSMCQD  VCEGMEYLER  NSFIHRDLAA  RNCLVNEAGV  VKVSDFGM
DSrc28C    MGLLLDMCIQ  VSKGMTYLER  HNYIHRDLAA  RNCLVGSENV  VKVADFGL

....Bmx    ILMWEVFSLG  KQPYDLYDNS  QVVLKVSQGH  RLYRPHLASD  TIYQIMYS
....Btk    VLMWEIYSLG  KMPYERFTNS  ETAEHIAQGL  RLYRPHLASE  KVYTIMYS
....Emt    VLMWEVFSEG  KIPYENRSNS  EVVEDISTGF  RLYKPRLAST  HVYQIMNH
....Tec    VLMWEIFTEG  RMPFEKNTNY  EVVTMVTRGH  RLHRPKLATK  YLYEVMLR
DSrc28C    VLMWEIFTCG  KMPYGRLKNT  EVVERVQRGI  ILEKPKSCAK  EIYDVMKL
```

Figure 1
(3/4)

```
SA  GLISRLKYPV  SQQ-NKNAPS  TAGLGYGSWE  IDPKDLTFLK  406
GG  GLVTRLRYPV  CFG-RQKAPV  TAGLRYGKWV  IDPSELTFVQ  367
AA  GLVTRLRYPV  STK-GKNAPT  TAGFSYDKWE  INPSELTFMR  270
SG  GL---ACRLK  SSPCDRPVPP  TAGLSHDKWE  IHPIQLMLME  332
..  ........pv  ..........  ..........  ..........  ...

PK  LVKFYGVCSK  EYPIYIVTEY  ISNGCLLNYL  RSHGKGLEP-  510
EK  LVQLYGVCTK  QRPIFIITEY  MANGCLLNYL  REMRHRFQT-  495
PK  LVQLYGVCLE  QAPICLVFEF  MEHGCLSDYL  RTQRGLFAA-  456
PK  LVQLYGVCTQ  QKPIYIVTEF  MERGCLLNFL  RQRQGHFSR-  359
PN  LVQLYGVCTK  HRPIYIVTEY  MKHGSLLNYL  RRHEKTLIGN  422

Autophosphorylation
TR  YVLDDQYVSS  VGTKFPVKWS  APEVFHYFKY  SSKSDVWAFG  599
SR  YVLDDEYTSS  VGSKFPVRWS  PPEVLMYSKF  SSKSDIWAFG  584
TR  FVLDDQYTSS  TGTKFPVKWA  SPEVFSFSRY  SSKSDVWSFG  545
AR  YVLDDQYTSS  SGAKFPVKWC  PPEVFNYSRF  SSKSDVWSFG  448
AR  YVLDDQYTSS  GGTKFPIKWA  PPEVLNYTRF  SSKSDVWAYG  514

CW  HELPEKRPTF  QQLLSSIEPL  REKDKH*.....675
CW  HEKADERPTF  KILLSNILDV  MDEES*......659
CW  KERPEDRPAF  SRLLRQLAEI  AESGL*......620
CW  QERPEGRPSF  EDLLRTIDEL  VECEETFGR*..527
CW  SHGPEERPAF  RVLMDQLALV  AQTLTD*.....590
```

TK

Figure 1
(4/4)

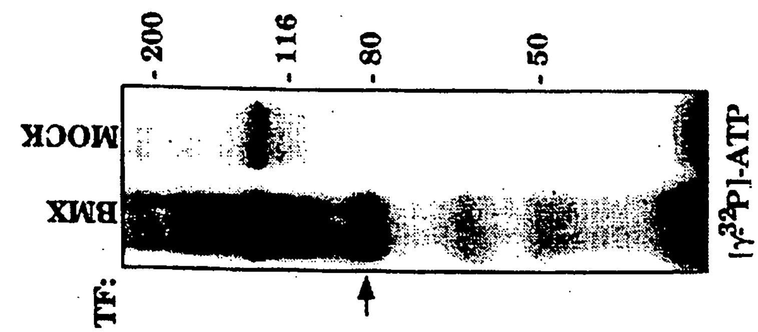


Figure 2D

Figure 2C

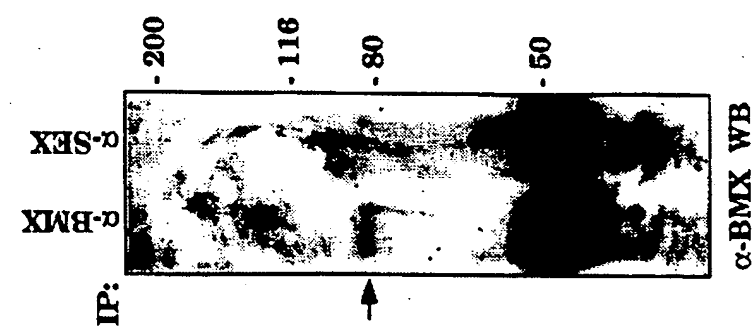


Figure 2B

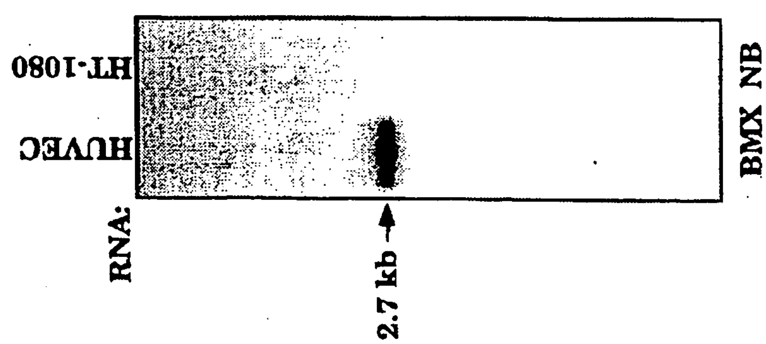


Figure 2A

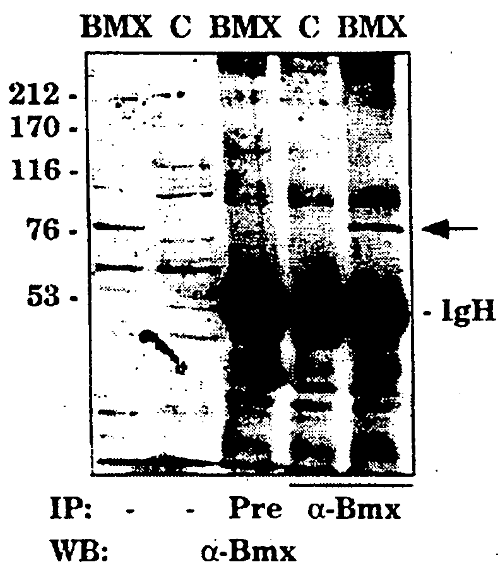
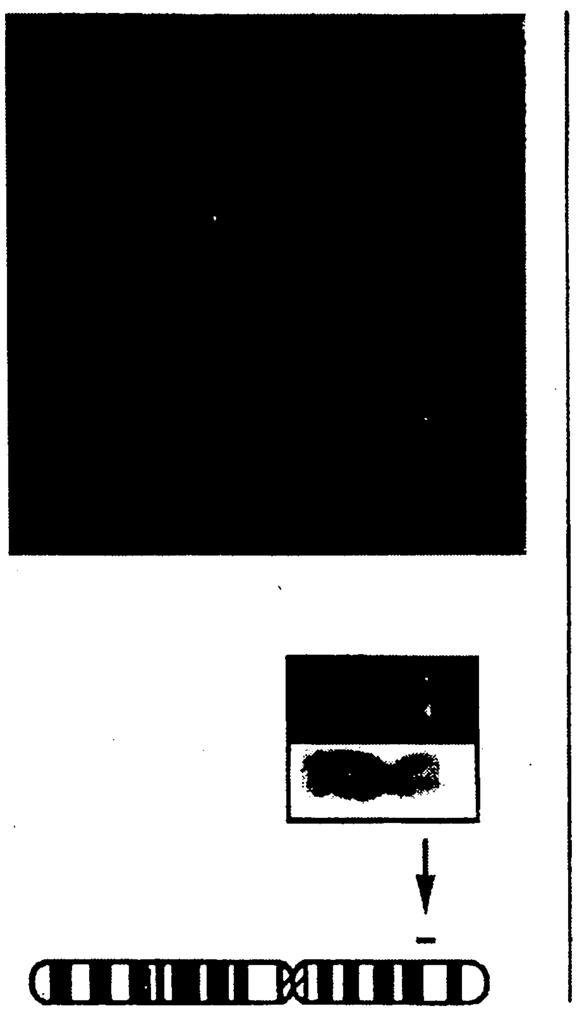


Figure 3



BMX: Xp22.2–21

Figure 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 95/00555

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12Q1/68 C07K16/40 C12N9/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, 1989 WASHINGTON US, pages 1603-1607, A. WILKS 'Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction' see the whole document ---	1-4
A	ONCOGENE (1994), 9(4), 1155-61 CODEN: ONCNES;ISSN: 0950-9232, April 1994 SAKANO, SEIJI ET AL 'Molecular cloning of a novel non - receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase)' see the whole document ---	1-5
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 1996	14.03.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Van der Schaal, C

Form PCT/ISA/210 (second sheet) (July 1992)

In ..tional Application No
PCT/FI 95/00555

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol. 361, 21 January 1993 pages 226-233, D. VETRIE ET AL 'The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases' cited in the application see the whole document ---	1-5
P,X	ONCOGENE (1994), 9(12), 3683-8 CODEN: ONCNES;ISSN: 0950-9232, December 1994 TAMAGNONE, LUCA ET AL 'BMX, a novel nonreceptor tyrosine kinase gene of the BTK¦ITK¦TEC¦TXK family located in chromosome Xp22.2' see the whole document -----	1-6

2